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Does change in precipitation magnitude affect the soil respiration response? A study on constructed invaded and uninvaded tropical grassland ecosystem

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ABSTRACT

Change in precipitation in tropics would have a severe consequence on soil CO_2 flux and on its major determinants like soil temperature and soil moisture. Variation in climatic condition also supports the establishment of invasive species in the area. In this communication an attempt is made to assess the impact of change in precipitation regime on soil CO_2 flux in a constructed tropical grassland with and without an invasive plant, *Hyptis suaveolens*. Three rainout shelters with manipulated precipitation doses (i) of 800 mm (20% the below average), (ii) 1100 mm (average) and (iii) 1600 mm (60% above the average) were established, along with one unsheltered plot (open C) receiving ambient precipitation. Each rain-out shelter containing three sub-treatments viz. (i) bare plots with no vegetation, (ii) uninvaded indigenous grassland plots (NIG), and (iii) grassland plots invaded with *H. suaveolens* (IG). Significant high soil CO_2 flux was found in plots receiving maximum precipitation (1600 mm) and low in plots receiving minimum precipitation (800 mm). Furthermore, vegetated plots (NIG and IG) exhibited higher soil CO_2 flux than the non-vegetated plots. Among the vegetated plots, IG plots receiving maximum precipitation (1600 mm) had highest soil CO_2 flux, followed by IG plots receiving 1100 mm, and 800 mm precipitation. Seasonal and monthly observations showed that maximum soil CO_2 flux occurred in rainy season, (in July), and minimum in winter season (in January). The important controlling factors for soil CO_2 flux were soil moisture, root biomass and soil temperature. In this study soil moisture explained 61% variability in the soil CO_2 flux. A linear combination of moisture and root biomass explained 74% variability in soil CO_2 flux, and when temperature was added in the multiple regression a total of 79% variability in soil CO_2 flux was accounted for. These findings would help us to understand the biological thresholds of tropical ecosystem related to precipitation regime. It will encourage researchers around the globe to develop ecosystem models, which would enable to forecast the impact of climate change on nutrient cycling.

1. Introduction

Rainfall in tropics is changing (Greve et al., 2014; Chadwick et al., 2016) and the changes are expected to be more extreme in future due to climate change (Malhi et al., 2008; Feng et al., 2013). Modelling approaches like Coupled Model Intercomparison Project Phase 5 (CMIP5) revealed that rainfall will decrease and dry period will increase in the tropics (Maloney et al., 2014; Duffy et al., 2015; Chadwick et al., 2016). Feng et al. (2013) also found an increase in inter-annual variability with equivocal response (increase and decrease both) regarding the rainfall magnitude and considerable uncertainty in the seasonal rainfall. Change in precipitation would be expected to affect the soil moisture regime and hence alter the soil carbon balance of an area via altered decomposition (Phillips et al., 2009, 2010; Gatti et al., 2014; Doughty et al., 2015), and hence soil CO_2 flux and nutrient cycling.

World ecosystems are highly sensitive to annual precipitation and its seasonality (Feng et al., 2013). Tropical ecosystems are in general considered as more sensitive towards disturbance because of their low adaptive capacity (Deutsch et al., 2008; Bouskill et al., 2016). Therefore, one can expect that change in rainfall regime would have severe consequence for regions with diverse climate and weather conditions having seasonal monsoonal rainfall pattern (Sathaye et al., 2006). The IPCC report (Flato et al., 2013) manifested that the Indian summer monsoon rainfall would increase in future as monsoonal circulation becomes weak. The change in rainfall pattern would not be uniform over the Indian sub-continent (Rathore et al., 2013). It therefore becomes imperative to understand how precipitation change would affect the natural ecosystems of the Indian subcontinent.

Indian grasslands are very heterogeneous and constitute around 24% of the total land area (Rawat and Adhikari, 2015). Grassland

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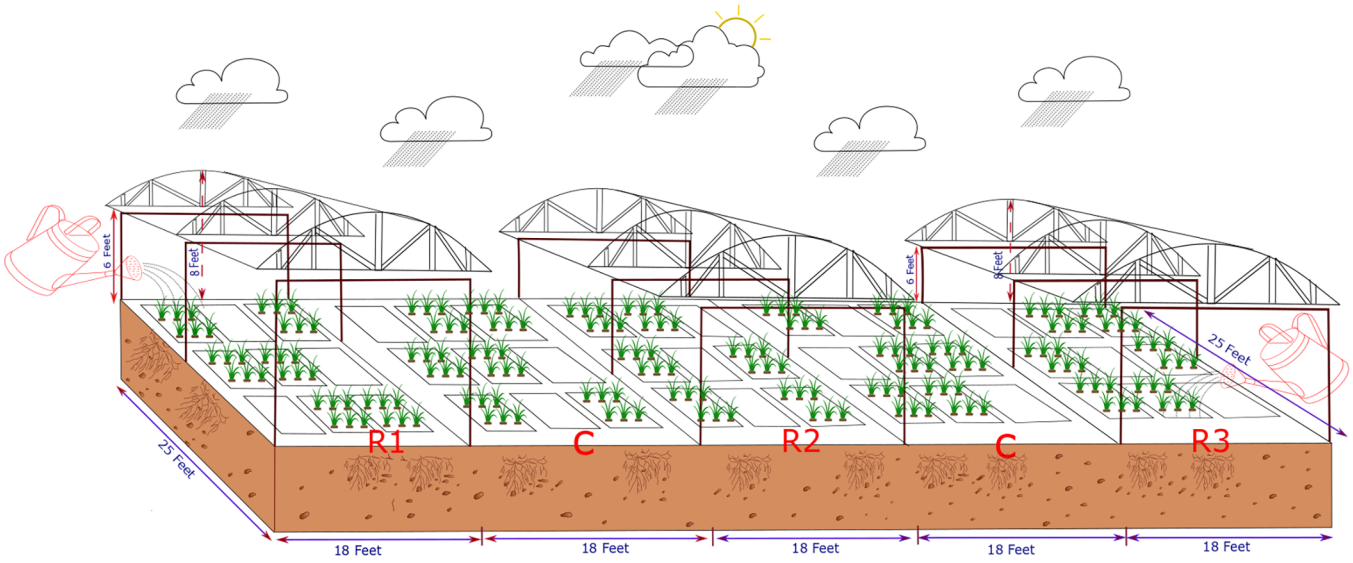

Fig. 1. Permanent rainout shelter construction and arrangements of the subplots at the experimental site. Three rainout sheltered and two ambient control plots were prepared alternately. Here we represent all plots where, R1 = 1600 mm, C = open C plot, R2 = 800 mm and R3 = 1100 mm. For more information refer to the main text.

ecosystem plays a significant role as a sink for atmospheric CO_2 by converting it into biomass and soil organic matter (Lal et al., 1998; Follett and Schuman, 2005; Gilmanov et al., 2010). Variability in temperature and precipitation indirectly and directly influence soil water dynamics of the grassland (Wilcox et al., 2017), and hence affect productivity and soil processes of these ecosystems (Wilcox et al., 2017). Long-term experiment using rainout shelter with manipulation of the precipitation can reveal important insights of ecosystem response to the changes in precipitation regime (Knapp et al., 2008).

Soil CO_2 flux is considered as a major carbon flux from the ecosystems, emitting 80 Pg C yr^{-1} (Chang et al., 2014; Singh et al., 2017). Soil CO_2 flux accounts for both non-metabolic fluxes and metabolic fluxes due to change in physical parameters and root or microbial activities, respectively (Adachi et al., 2009). Soil respiration is expected to be affected by soil temperature and soil moisture in the tropical regions (Sotta et al., 2006). It is also affected by fine root biomass (Silver et al., 2005; Adachi et al., 2006), microbial community (Srivastava et al., 2016; Singh et al., 2017), above-ground net primary productivity (ANPP) (Raich and Tufekciogul, 2000), substrate availability (Cleveland et al., 2010; Vicca et al., 2014; Srivastava et al., 2016; Singh et al., 2017) and vegetation cover (Raich and Tufekciogul, 2000). In addition, soil CO_2 flux also varied spatially across natural ecosystems (Singh et al., 2017) and may contribute positively to the climate change (Chang et al., 2014).

As far as biodiversity is concerned, invasive species are the biggest threat (Simberloff, 1995; Simberloff et al., 2013). *Hyptis suaveolens* (L.) Poit. is one of the emerging invaders of tropical and sub-tropical regions (Wulff and Medina, 1971; Sarmiento, 1984; Afolayan, 1993) including different parts of India (Wealth of India, 1959; Yoganarasimhan, 2000; Sharma et al., 2009). Invasive species spread faster than native species (Padalia et al., 2015) and affect productivity and species composition of an area (Hulme et al., 2013). Climate change may facilitate establishment of invasive species in the area and hence it can disturbs the equilibrium of an ecosystem (Walther et al., 2009).

Numerous rain manipulated studies are available for the temperate world (Harper et al., 2005; Lin et al., 2016; Miao et al., 2017) but in the tropical region rain manipulated research is lacking and those involving grassland ecosystems are rare. The aim of the present study is to assess the impact of change in precipitation magnitude on the soil CO_2 flux in a constructed grassland with and without an invasive plant (*Hyptis suaveolens*). Additionaly, we test the hypothesis that the presence of invasive plants together with alteration in rainfall would lead to a significant alteration in soil CO_2 flux.

2. Materials and methods

2.1. Study sites

The study was performed in the experimental plots of the Botanical Garden of the Banaras Hindu University (25°16′3.3″ N and 82°59′22.7″ E), Varanasi, Uttar Pradesh, India. Climate is monsoonal with an annual rainfall of about 1100 mm, of which 80% is received between June and September. The year is characterized by three seasons, viz., a hot summer (April to June), a warm rainy season (July to September), and a cold winter (November to February). The mean monthly temperature ranges from a minimum of 13 °C during winter to a maximum of 35 °C during the summer season. The locality thus, experiences a strong seasonality making the rainy season as the grand growth period for plants and the summer season as the most hostile to plant growth. The soil is deep, pale brown in colour and silty loam in texture, and is characterized as inceptisol.

2.2. Experimental design

The experiment was simultaneously conducted in both rainout shelters (8T, 11T, 16T plots) and in unsheltered plots (Open C). Both rainout shelter and unsheltered control plots were further divided into nine subplots (1 × 1 m). These subplots were randomly assigned to each of the three treatments (i) bare plots with no vegetation (BP), (ii) non-invaded indigenous grassland plots (NIG) and (iii) grassland plots invaded with *H. suaveolens* (IG). Each treatment thus was replicated thrice i.e., three BP, three NIG and three IG plots. The vegetation of the BP was removed from time to time. In NIG plots patches of indigenous plants were planted. These patches were collected from an adjacent native grassland. Whereas in IG plots, together with patches of indigenous plants *H. suaveolens* was established by transplanting 15 seedlings per meter square. The density of *H. suaveolens* was higher than its natural density in invaded grassland (8 per m^2). The number of transplanted seedlings was kept higher than found in the field to permit destructive sampling. The observations were taken after one year of the establishment of the experimental plots.

The designed rainout shelter is of static type and is well-ventilated

(Fig. 1). The rainout shelter was properly roofed with a transparent sheet of polyethene, so that the transmittance of natural light would take place without hindrance. And being well ventilated the plots experienced conditions similar to the unsheltered area as for as air temperature and relative humidity are concerned. The experimental plots under the rainout shelter were sprinkled with a fixed amount of water on fixed days throughout the year.

2.3. Manipulated precipitation dose

The amount of water added to the average precipitation treatment was based on a 12-year data of precipitation records (2001–2012), available from the previously recorded daily meteorological data at IMD unit, Department of Agronomy, Institute of Agriculture Sciences, BHU. We also calculated the average number of days on which rain occurred in each month from the 12-year rainfall dataset. With these calculations, we prepared an entire calendar for the total number of watering days for the years 2014 and similarly followed for 2015 and 2016. All plots were watered manually by groundwater using a sprinkler. The volume of water to be sprinkled was adjusted for the surface area across experiments.

As from the 12-year dataset, minimum rainfall of 670.8 mm occurred in the year 2004, and the maximum 1336.5 mm occurred in 2002, and the average precipitation was 1100 mm per year. Accordingly, three precipitation doses were applied viz. (i) 800 mm (8T, 20% below the average) (ii) 1100 mm (11T, average) and (iii) 1600 mm (16T, 60% above the average). As per records of the Indian Metrological Department, the maximum decrease in rainfall in India was by 20% up to 2010 (Rathore et al., 2013). In one treatment we provided 1600 mm rain, i.e., 60% above the 12-year average rainfall, as IPCC 2013 reports that monsoonal rain will increase in future. In India, 80% of rain occurs in four months i.e., rainy season, so, we adjusted the rain doses accordingly to maintain the seasonality. Summary of rainfall amount and events in both ambient condition and rain manipulated study are given in Table 1.

2.4. Soil CO_2 flux measurement and other related properties

Sampling for soil CO_2 flux, soil temperature and soil moisture were performed monthly from Dec 2015 to Dec 2016 and these variables were also recorded during peak season of winter (January), summer (June) and rainy (July) respectively. Soil CO_2 flux was measured by the closed chamber method using an IRGA (Infra Red Gas Analyzer) with the help of Licor LI-6400–09 (Licor Inc., Lincoln NE, USA). Soil CO_2 flux collar (a polyvinyl chloride cylinder, 6 cm height, 11 cm in internal diameter) was placed permanently into the soil at a depth of 2 cm in the middle of each nine subplots to avoid edge effect. Readings were taken from the same collar, and any vegetation occurring inside the collar was removed 24 hrs before taking the flux reading. Flux measurements were made between 09.00 and 12.00 h.

Soil temperature was measured simultaneously with the flux by Li-Cor temperature probe (Licor LI-6400-09, Licor Inc., Lincoln NE, USA). Soil moisture content at 0–10 cm depth was measured monthly by gravimetric method taking soil samples from each of the nine subplots on the same day that soil CO_2 flux was measured. Root biomass of the NIG grassland was determined by sampling monoliths of 15 × 15 × 15 cm in June, July, September and December from each 1 × 1 m subplot. Monoliths were washed with fine jet of water using mesh screens. *Hyptis* root biomass was determined by randomly picking one *Hyptis* individual from each 1 × 1 m IG plot in the month of June, July, September and December.

ANPP of the NIG grassland was determined by harvesting all above-ground biomass at the end of the growing season (September) using 25 × 25 cm quadrats in the year 2016. In IG plots, above-ground biomass of *H. suaveolens* was measured by harvesting three individuals of the species in September (reproductive stage). All the samples of grassland and *Hyptis* were dried in the oven at 70 °C for 72 h to constant weight.

Table 1
Summary of rainfall quantity and events occurred in the ambient control condition along with the quantity of rain doses given in rainout sheltered treatment plots (2014–2016).

Year	Rainy season (June–Sept)		Non-rainy season (Oct–May)		Total annual	
	Amount (mm)	Rain events	Amount (mm)	Rain events	Amount (mm)	Rain events
Open plots (open C)						
2014	688	41	244.7	23	932.7	64
2015	857.3	40	152.1	17	1009.4	57
2016	1185.5	56	61.5	12	1247	68
Treatment plots						
2014–2016	640	56	160	15	800 (8T)	71
	880	56	220	15	1100 (11T)	71
	1280	56	320	15	1600 (16T)	71

2.5. Statistical analysis

Statistical analysis was conducted by using with the help of SPSS (SPSS Inc. Version 16). Data was analysed by Multivariate Analysis of Variance (MANOVA) to determine the effect of precipitation magnitude and vegetation type on soil CO_2 flux and its determinants (soil moisture and soil temperature). Post hoc (Tukey) test was applied to differentiate between the means. Pearson correlation and regression analysis were performed to see the relationships among attributes such as soil CO_2 flux, soil temperature, soil moisture, root biomass and ANPP. Bray-Curtis similarity cluster analysis was performed to group different precipitation treatment plots on the basis of similarity of soil CO_2 flux and soil moisture data by using BioDiversity Pro.

3. Results

3.1. Soil moisture and soil temperature

In sheltered plots, soil moisture and soil temperature varied significantly with the years (Y), seasons (S), precipitation (P); IG, NIG and B vegetation treatments (T) and with their interactions (Supplementary Table 1). While in ambient unsheltered plots (Open C), soil moisture and soil temperature varied significantly with Y, S and with their interactions (Supplementary Table 2). However, soil temperature varied significantly with T also and soil moisture does not (Supplementary Table 2).

Soil moisture exhibited strong seasonal variation with a maximum in the rainy season than in winter and summer season in all manipulated precipitation plots (Fig. 2). Soil temperature also showed seasonality with higher value in summer than in rainy and winter season (Fig. 3). In comparision with open C, soil moisture content of 8T plots decreased by 43% and 54% in winter and rainy seasons respectively, but increased by 61% in the summer season. In 11T plots soil moisture increased by 12% and 76% in winter and summer season, respectively, but decreased by 8% in the rainy season. In 16T plots soil moisture increased by 56% and 9% in summer and rainy season, but decreased by 12% in the winter season.

Soil temperature when compared with open C, in 8T plots increased by 30%, 5% and 4% in winter, summer and rainy season, respectively. In 11T plots soil temperature decreased by 4% and 4% in summer and rainy season respectively, but increased by 27% in winter season. In 16T plots soil temperature decreased by 0.5% and 0.4% in summer and rainy season, respectively, but increased by 27% in winter season.

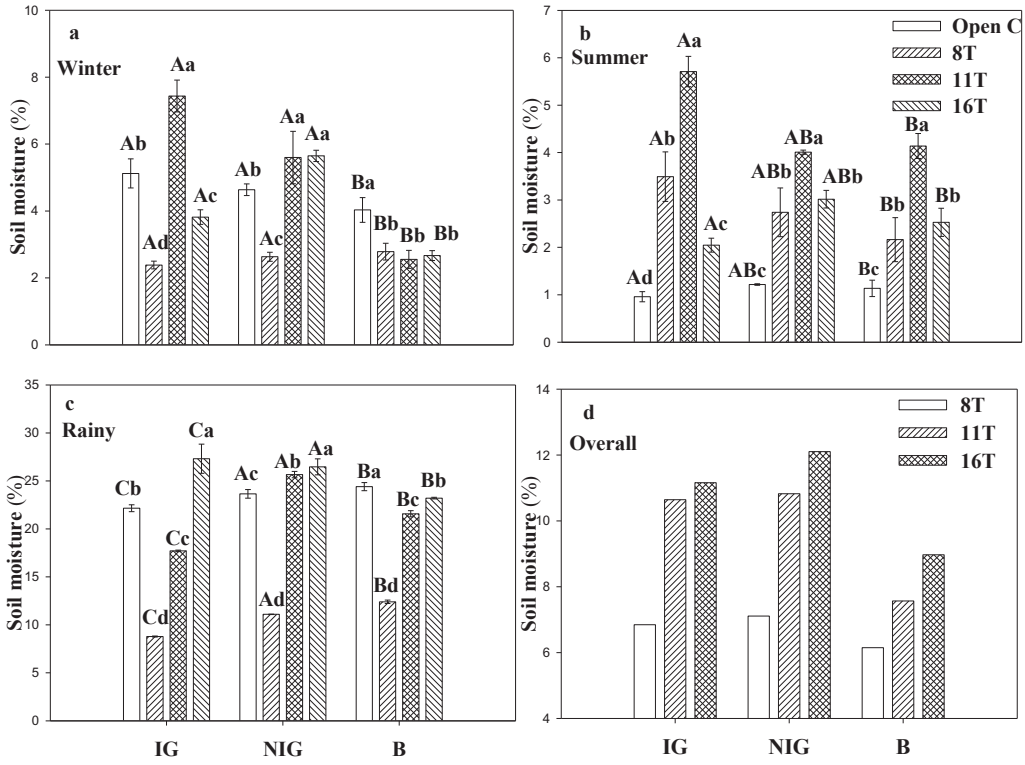

Fig. 2. Soil moisture among different precipitation (C, 8T, 11T, 16T) and vegetation treatments (IG, NIG, B). (a) Winter season (seasonal data of 2015–16 were pooled), (b) summer season (seasonal data of 2015–16 were pooled), (c) rainy season (seasonal data of 2015–16 were pooled) and (d) average soil moisture dataset of entire 13 months under sheltered plots from Dec-15 to Dec-16 (See Supplementary Table 3 for MANOVA). Bars affixed with different combinations of letters are significantly different from each other ($P < 0.05$). The uppercase letters represent vegetation treatments and lower case letters represent precipitation. Values are ± 1SE.

Precipitation magnitude effectively altered the soil moisture content of sheltered plots (Supplementary Tables 1, 3; Fig. 2d). When considering both vegetated (IG and NIG) and non-vegetated (B) treatment plots, soil moisture content in rainy season was highest in 16T plots and lowest in 8T plots, the value showed general trend as 16T > 11T > 8T (Fig. 2). However, in both winter and summer season IG plots showed the highest soil moisture in 11T rather than 16T (Fig. 2).

Treatments (IG, NIG and B) had a substantial effect on soil moisture in all sheltered precipitation plots (8T, 11T, 16T) (Supplementary Table 1), however it did not affect open C plots (Supplementary Table 2). Soil moisture content in IG plots showed significantly higher value than that of NIG and B plots in both winter and summer season (Fig. 2). However in rainy season, soil moisture followed different trend i.e., was showing significantly higher value in NIG than B and IG plots (Fig. 2). Soil temperature in treatment plots followed the trend as B > NIG > IG in summer and rainy seasons, respectively, except in winter season (Fig. 3).

3.2. Soil CO_2 flux

In sheltered and open C plots, soil CO_2 flux varied significantly with the S, T and with their interactions (Supplementary Tables 1, 2). However, soil CO_2 flux in sheltered and open C plots also varied significantly with P and Y, respectively (Supplementary Tables 1, 2).

Like soil moisture content, soil CO_2 flux also exhibited strong seasonality with higher value in rainy season than in summer and winter seasons (Fig. 4). When compared with open C, in 8T plots, soil CO_2 flux decreased by 27%, 4% and 47% in winter, summer and rainy season, respectively. In 11T plots soil CO_2 flux decreased by 23% and 36% in winter and rainy season, respectively, but increase by 3% in the summer season. In 16T plots soil CO_2 flux increased by 13% and 29% in winter and summer season, respectively, but decreased by 13% in the rainy season.

Soil CO_2 flux in all treatment plots (IG, NIG and B) was highest in 16T, than in 11T and 8T irrespective of the season (Fig. 4). IG plots had significant higher soil CO_2 flux than that of NIG and B plots in all three seasons showing general trend as IG > NIG > B (Fig. 4).

3.3. Aboveground net primary productivity (ANPP) and Hyptis biomass

ANPP differed significantly among grassland plots (NIG) ($F = 5.442$, $P = 0.025$). Soil CO_2 flux and aboveground net primary productivity (ANPP) of the NIG responded similarly to the precipitation (Figs. 4, 5) i.e., highest ANPP in 16T followed by 11T and 8T (Fig. 5). With the increase in precipitation, above ground biomass of *Hyptis* ($F = 10.602$, $P = 0.004$) (Fig. 5) and root biomass also increased (Table 2) similarly as soil CO_2 flux (Fig. 4).

3.4. Patterns of temporal variation

Soil CO_2 flux largely followed the trend of soil moisture in all treatments (IG, NIG and B) and precipitation plots (Fig. 6), showing maximum soil CO_2 flux in July which is the hottest month of the rainy season when temperature is optimum and soil moisture content is high (Fig. 6). The bare (B) plots showed another peak of soil CO_2 flux in October as soil moisture was higher in this month (Fig. 6).

Soil temperature exhibited a bell-shaped curve in all treated plots (Fig. 6), showing highest values in April, May, June and July (Fig. 6). Among the precipitation plots, soil temperature was highest in 8T plot, i.e. in plots receiving minimum precipitation (Fig. 6). Soil temperature followed the trend 8T > 11T > 16T in all treatment plots (Fig. 6).

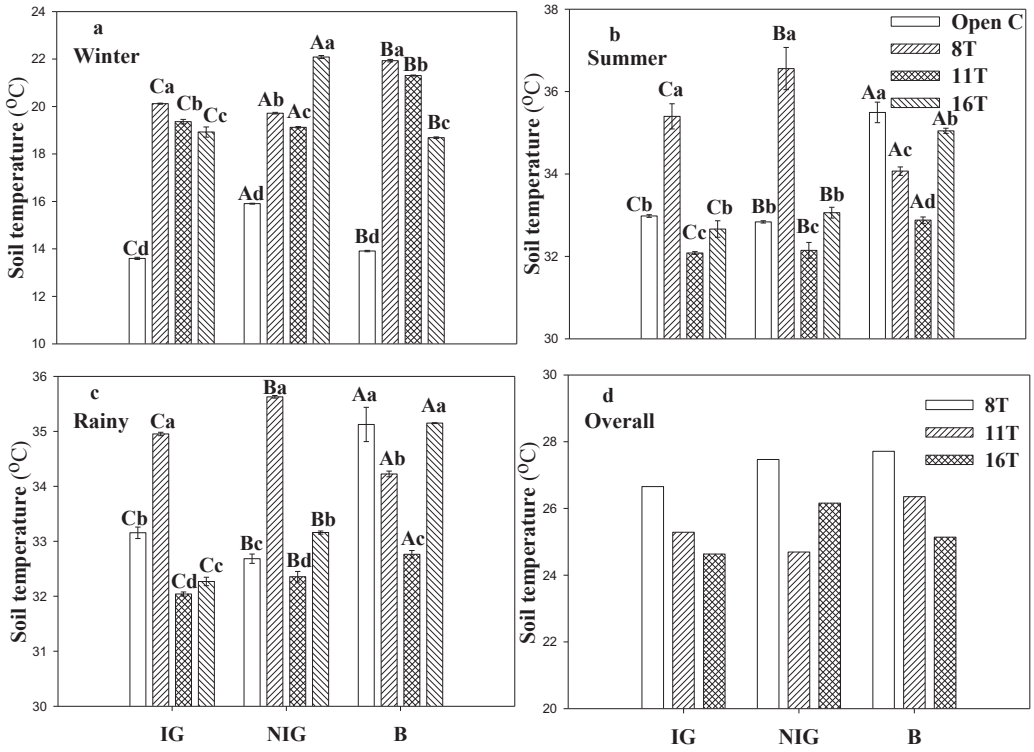

Fig. 3. Soil temperature among different precipitation (C, 8T, 11T, 16T) and vegetation treatments (IG, NIG, B). (a) Winter season (seasonal data of 2015–16 were pooled), (b) summer season (seasonal data of 2015–16 were pooled), (c) rainy season (seasonal data of 2015–16 were pooled) and (d) average soil temperature dataset of entire 13 months under sheltered plots from Dec-15 to Dec-16 (See Supplementary Table 3 for MANOVA). Bars affixed with different combinations of letters are significantly different from each other ($P < 0.05$). The uppercase letters represent vegetation treatments and lower case letters represent precipitation. Values are ± 1SE.

3.5. Factor responsible for soil CO_2 flux

Soil moisture had a linear relationship with soil CO_2 flux (Fig. 7) and was a better predictor of soil CO_2 flux than soil temperature (Table 3). In low precipitation, soil temperature and soil moisture both govern the rate of soil CO_2 flux (Table 3), however, in high precipitation, only soil moisture governs (Table 3).

Soil CO_2 flux was linearly related to soil moisture (Fig. 7) and root biomass (Fig. 8a). When the data of SM, RB and ST were regress to predict soil CO_2 flux, we found that soil moisture is the most important governing factor for soil CO_2 flux ($R^2 = 0.61$) (Table 4). A linear combination of root biomass and soil moisture increased the R^2 value by 21%, and when soil temperature was included in the combination the R^2 value was increased by 30% (Table 4). Soil CO_2 flux also showed a linear relationship with ANPP of the NIG (Fig. 8b) and the above ground *Hyptis* biomass of the IG plots (Fig. 8c).

Seasonality has a strong influence over the relationship of soil moisture and soil temperature with soil CO_2 flux; in the winter season soil moisture was negatively correlated with soil CO_2 flux in 8T ($r = -0.55$, $P < 0.05$), 11T ($r = -0.73$, $P < 0.01$) and 16T (-0.72, $P < 0.01$) precipitation. While soil temperature was significantly positively correlated with soil CO_2 flux in open C ($r = 0.71$, $P < 0.05$). In summer season, soil temperature was significantly negatively correlated with soil CO_2 flux in open C ($r = -0.53$, $P < 0.05$), 8T ($r = -0.41$, $P < 0.05$), 11T ($r = -0.69$, $P < 0.01$) and 16T ($r = -0.69$, $P < 0.01$). In rainy season, soil moisture had a significant positive relationship with soil CO_2 flux in 16T ($r = 0.48$, $P < 0.05$), while soil temperature was significantly positively related with soil CO_2 flux in 8T ($r = 0.48$, $P < 0.05$) and 16T ($r = 0.85$, $P < 0.01$).

In our case we also tried to group the different precipitation plots (C, 8T, 11T and 16T) with their soil moisture and soil CO_2 flux data by using Bray-Curtis similarity cluster analysis. On the basis of soil moisture, these precipitation plots were divided into three groups; the first group comprised open C and 16T plots; the second group 11T plots and the third group 8T plots (Fig. 9). On the basis of soil CO_2 flux, precipitation plots were clustered into two groups, the first group comprised open C and 16T plots and the second group 8T and 11T plots (Fig. 9).

4. Discussion

Change in the magnitude of rainfall, as expected under climate change scenarios, effectively altered the soil moisture content in the constructed plots, and had a huge influence on the productivity (ANPP) and soil processes of the constructed grassland. For instance, soil CO_2 flux and ANPP increased with an increase in the precipitation and decreased with reduced precipitation. ANPP is the amount of aboveground carbon accumulated in any ecosystem over a particular time period. Other study further showed that soil CO_2 flux and ANPP are positively related with each other as also reported by Raich and Tufekciogul (2000), Harper et al. (2005), Vargas et al. (2010), and Thomey et al. (2011). Increased in soil CO_2 flux in the 16T plots in the uninvaded grassland (NIG) could be due to increased productivity.

Invasive species like *H. suaveolens* are opportunistic and need to be studied in this era of climate change for response and mitigation assessment. One such modelling approach had been taken up by Padalia et al. (2014), indicating, it is likely that *H. suaveolens* will grow better in the wetter region of the world. Our result also shows that, *H. suaveolens* would expand its climatic envelope with the increase in precipitation. For example, in the present study, growth of *H. suaveolens* gets affected by both precipitation received in sheltered plots and with the seasonality. Thus, the growth of *H. suaveolens* was higher in plots receiving

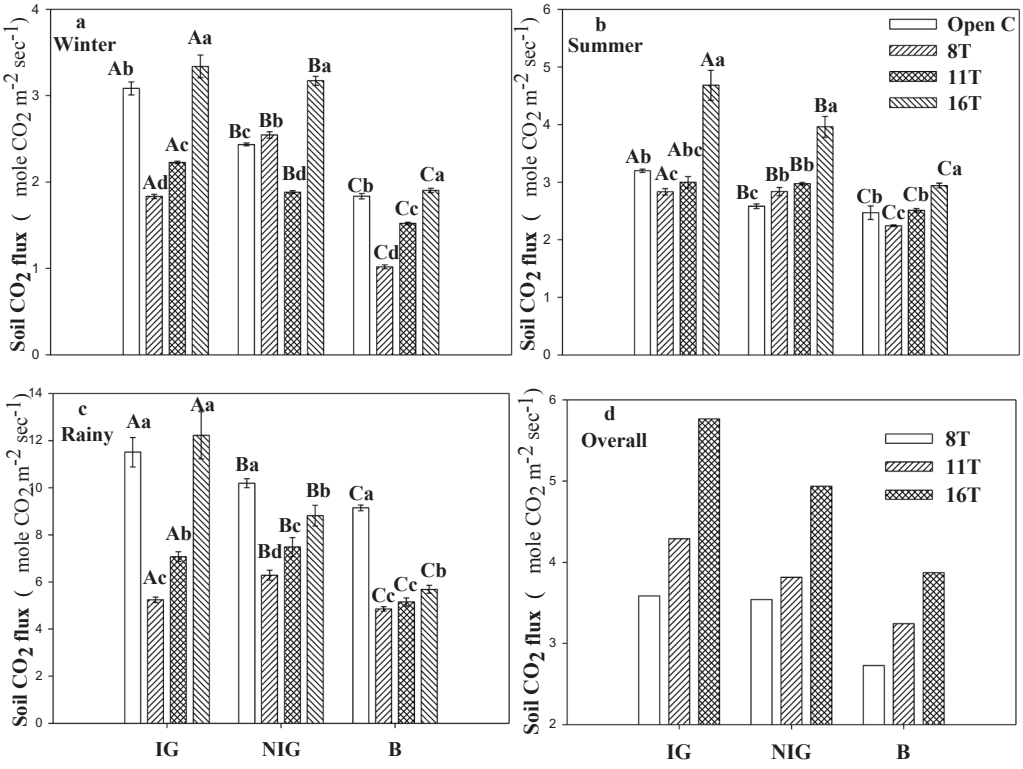

Fig. 4. Soil CO_2 flux among different precipitation (C, 8T, 11T, 16T) and vegetation treatments (IG, NIG, B). (a) winter season (seasonal data of 2015–16 were pooled), (b) summer season (seasonal data of 2015–16 were pooled), (c) rainy season (seasonal data of 2015–16 were pooled) and (d) average soil CO_2 flux dataset of entire 13 months under sheltered plots from Dec-15 to Dec-16 (See Supplementary Table 3 for MANOVA). Bars affixed with different combinations of letters are significantly different from each other ($P < 0.05$). The uppercase letters represent vegetation treatments and lower case letters represent precipitation. Values are ± 1SE.

maximum precipitation (16T) in rainy season, and was low in plots receiving minimum precipitation (8T) in the summer season.

H. suaveolens like other invasive species will grow faster than the native species and would affect the biodiversity and soil moisture content of the newly invaded area, resulting in a severe impact on the ecology (Blank and Morgan, 2014; Nielsen et al., 2014, Afreen et al., 2017). Faster growth of *H. suaveolens* in rainy season affects soil moisture content in 8T, 11T, i.e. soil moisture was lower in invaded plots compared to uninvaded plots. However, *H. suaveolens* does not grow properly in winter and summer showing no effect on soil moisture content. Moreover, in 16T plots conditions are favourable for the growth throughout the year, leading to decreased soil moisture content in invaded plots (IG) also in winter and summer. Therefore, vegetation has to be managed such that incidence of invasive species is minimized and the vegetation is maintained under natural conditions.

Vegetation influences the climatic conditions of an area by altering soil properties and related processes (Adams et al., 2012; Zeppel et al., 2014; Bonan, 2015). In the present, the soil CO_2 flux was higher in the vegetated plots (IG and NIG) as compared to the non-vegetated bare plots (B). It reflects that the root and microbial respiration (metabolic respiration) contributes a major portion to the evolution of soil CO_2. Moreover, when we consider only vegetated plots, the effect of invasion on the grassland is quite apparent, showing higher soil CO_2 flux in invaded grassland (IG) than in uninvaded grassland (NIG), as there occurred a positive co-relation between root biomass and soil CO_2 flux; and between *Hyptis* biomass and CO_2 flux (Fig. 9). The study also highlights the importance of root biomass in the emission of soil CO_2. Raich and Tufekciogul (2000) reported that the effect of vegetation on soil CO_2 flux was possibly as a result of change in soil microclimate, the quantity and quality of detritus and rates of root respiration.

Monsoon, which lasts from July to September, plays a crucial role in the functioning of dry tropical grassland. Impact of seasonality on the property and processes of the soil was observed in many researches (Phillips et al., 2009, 2010; Feng et al., 2013; Gatti et al., 2014; Doughty et al., 2015). In present study, soil CO_2 flux and its determinants also showed strong seasonality. For an instance, soil CO_2 flux was maximum in rainy season as both soil temperature and soil moisture favours the growth of microorganisms and plants (Gupta and Singh, 1981). Minimum soil CO_2 flux was found in the winter season (November to February) as low temperature and soil moisture occurred in this season (Gupta and Singh, 1981). Soil CO_2 flux was intermediate in summer season (April to June) due to high temperature and low moisture conditions (Gupta and Singh, 1981).

Soil CO_2 flux showed strong seasonal pattern with maximum values after the onset of monsoon, i.e. in July and minimum in December. This sudden peak of soil CO_2 flux in July after hot summer is similar to the pulse events in arid and semi-arid ecosystems in which large soil CO_2 flux are observed after first rainfall subsequent to drought (Xu et al., 2004). Factors responsible for this pulse event in arid ecosystems are known as Birch effect. The study showed that the phenomenon like Birch effect also occurs in tropical soil (Liptzin and Silver, 2015), when microbial, and mineralization activities increased with the rain (Singh et al., 1989; Jha et al., 1996; Singh and Kashyap, 2007). Other studies have also shown that CO_2 pulses might occur due to increased availability of organic substrate because of the breakdown of soil aggregates (Manzoni et al., 2014). With the onset of the monsoon, soil

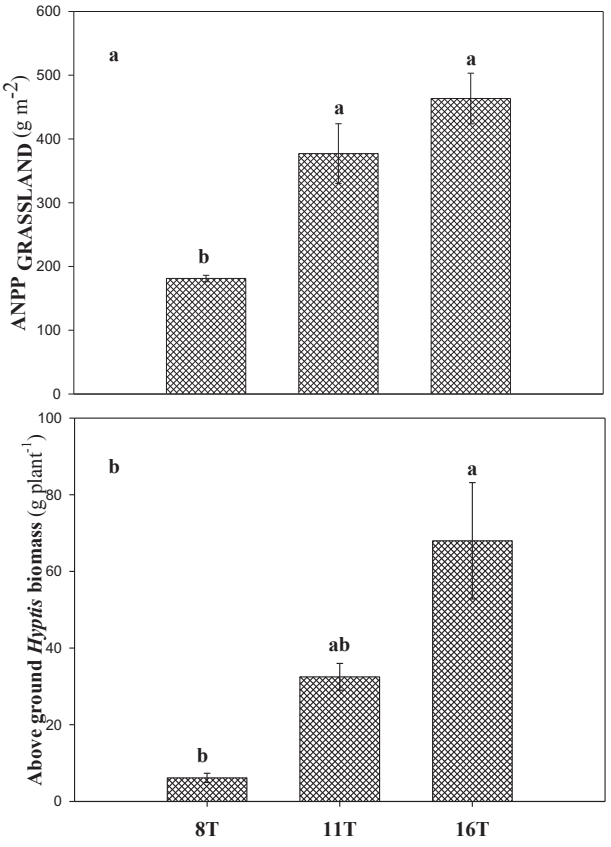

Fig. 5. (a) Represents ANPP of uninvaded grassland (NIG) and (b) represents above ground *Hyptis* biomass in invaded grassland plots (IG) under different precipitation regime.

microorganisms which are present in dormant form become active (Singh et al., 1989), and plant growth starts. From July–September (rainy season) we have full-fledged growth of vegetation and huge microbial activities. In September, we have a rich floral diversity hence contributing large emission of soil CO_2.

Biological origin of soil CO_2 flux is also confirmed by the bare plots in which soil CO_2 flux followed the same trend as vegetated plots, that is maximum soil CO_2 flux in July. However, the origin of soil CO_2 flux in bare and vegetated plots must be different. The metabolic soil CO_2 flux can be divided into two parts autotrophic and heterotrophic soil respiration (Hanson et al., 2000). In bare plots soil CO_2 flux is chiefly contributed by heterotrophic respiration (Chang et al., 2014) fuelled by stored organic matter. In vegetated plots both autotrophic and heterotrophic respiration contributes to soil CO_2 flux (Knorr et al., 2005; Heinemeyer et al., 2007; Chang et al., 2014).

Interaction of soil moisture and soil temperature would have an important consequence on soil CO_2 flux in the disturbed precipitation distribution due to global warming (Rastogi et al., 2002). Present study revealed, in low precipitation condition, soil temperature and soil moisture primarily regulates soil CO_2 flux while in high precipitation only soil moisture act in to play (Table 3). Overall, soil CO_2 flux was chiefly influenced by soil moisture in open C, 8T, 11T and 16T plots. Our study shows that in tropical grassland ecosystems soil moisture mainly governs the flux of soil CO_2. We also found linear relationship instead of the hump-shaped curve between soil CO_2 flux and soil moisture in all precipitation treatment plots (8T, 11T, 16T) including open C. This may be because the maximum soil moisture concentration present in the rain-out experiment was in the range of 12 (8T) − 27% (16T) in the rainy season, which did not lead to the anaerobic condition triggering reduction in soil CO_2 flux (Chang et al., 2014).

We emphasize that tropical ecosystems are entirely seasonal and shifts in the precipitation regime will lead to devastating effect on the biodiversity and productivity of the area. In our study, carbon accumulation (ANPP) in tropical grassland ecosystem increases with the increase in the precipitation. However, we also found increase in the soil CO_2 flux, thus positive feedback to the carbon flux cycle. Moreover, to predict any conservation strategy a detailed long-term study is needed related to the behaviour of forbs and graminoid species in relation to climate change, more specifically. Invasive species like *H. suaveolens* are opportunistic and as our results indicate, *H. suaveolens* would expand its climatic envelope with the increase in precipitation. Invasive *H. suaveolens* will grow faster than the native species and would affect the biodiversity of the newly invaded area. To mitigate the effect of *H. suaveolens* invasion, one of the best strategy is to eradicate it completely manually by digging its rhizomes present in the soil, so, that it would not grow back as monsoon appears. Moreover, having large protected areas and managing them would help us to retain the biodiversity of the area, which would help tropical grassland to maintain their resilience.

Overall, in present study, alteration of precipitation magnitude and related change in soil moisture content significantly changed soil temperature and soil CO_2 flux. The ANPP, root biomass and soil CO_2 flux behave similarly to the change in precipitation regime in constructed uninvaded grassland. Moreover, increase in precipitation would further increase the risk of invading species which prefer wetter area for their growth such as *H. suaveolens*, supplementing more soil CO_2 flux to the environment. In dry tropical environment soil CO_2 flux and its determinants are deeply affected by seasonality. Hence, our study indicates the sensitivity of Indian grassland to the change in rainfall quantity. The increase in precipitation quantity would shift dry tropical grassland ecosystem towards the net source of biogenic soil CO_2 flux. However, a decrease in precipitation would release less soil CO_2 flux to the environment than average. Moreover, to validate the function of grassland ecosystem as carbon sink in decrease precipitation, detailed studies of carbon cycle are requires at regional and global level. In addition, this study would help to develop regional climate models to get appropriate results related to climate change in future for the Indian subcontinent and can be incorporated into the Global circulation models (GCMs) as suggested by Hannah et al. (2002).

Table 2
Root biomass of grassland in four months under sheltered plots.

Root biomass (g m^{-2})	June	July	September	December
8T	29.5 ± 7.85^{b}	42.3 ± 1.01^{ab}	50.7 ± 1.41^{a}	49.4 ± 0.48^{a}
11T	54.9 ± 0.90^{b}	76.6 ± 9.04^{ab}	105.8 ± 12.85^{a}	86.6 ± 5.86^{ab}
16T	79.5 ± 1.13^{b}	115.5 ± 2.45^{a}	130.7 ± 9.90^{a}	121.6 ± 3.83^{a}

Different combinations of letters are significantly different from each other ($P < 0.05$).

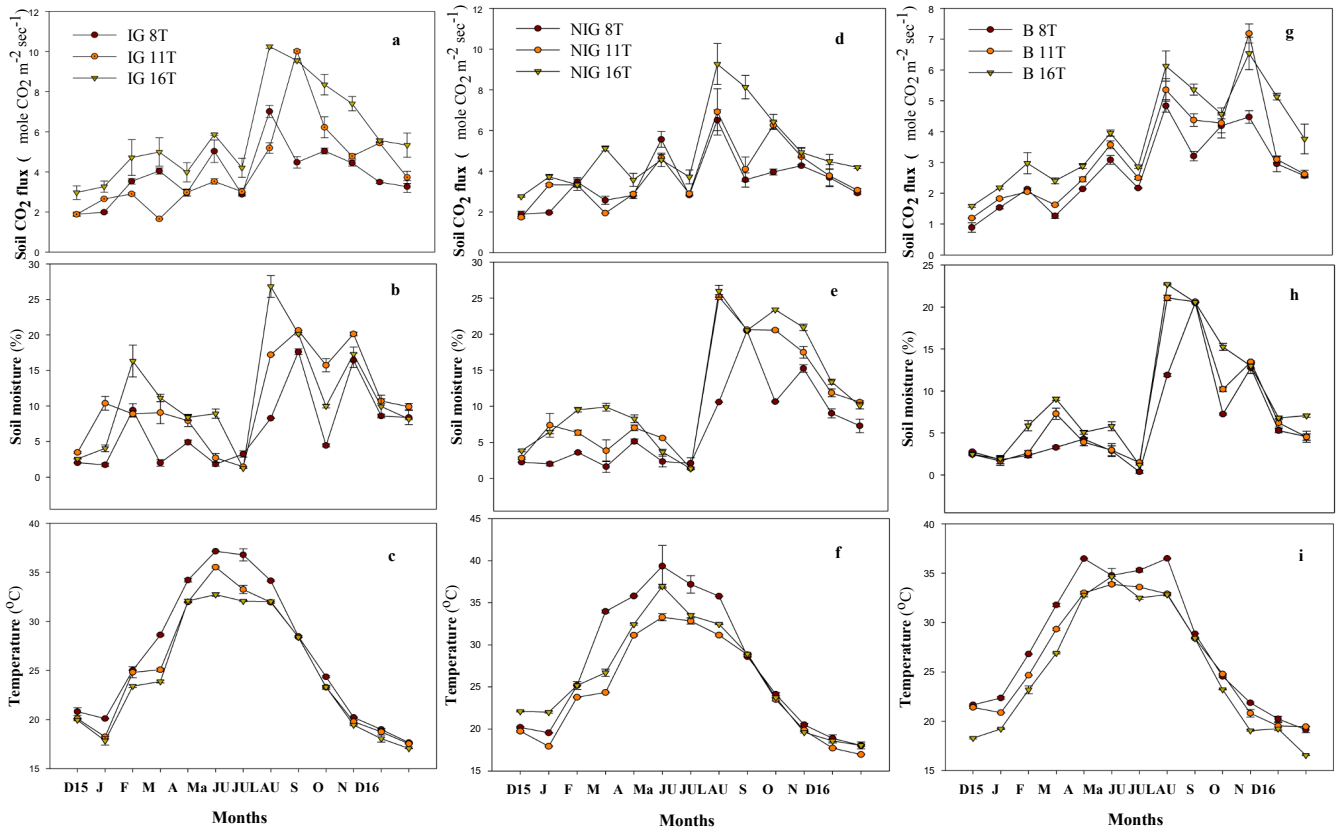

Fig. 6. Mean monthly soil CO_2 flux, soil moisture and soil temperature under different precipitation regime in *Hyptis* invaded grassland (IG) (a, b, c), uninvaded grassland (NIG) (d, e, f) and bare plots (B) (g, h, i). Data of entire 13 months were shown (Dec-15 to Dec-16).

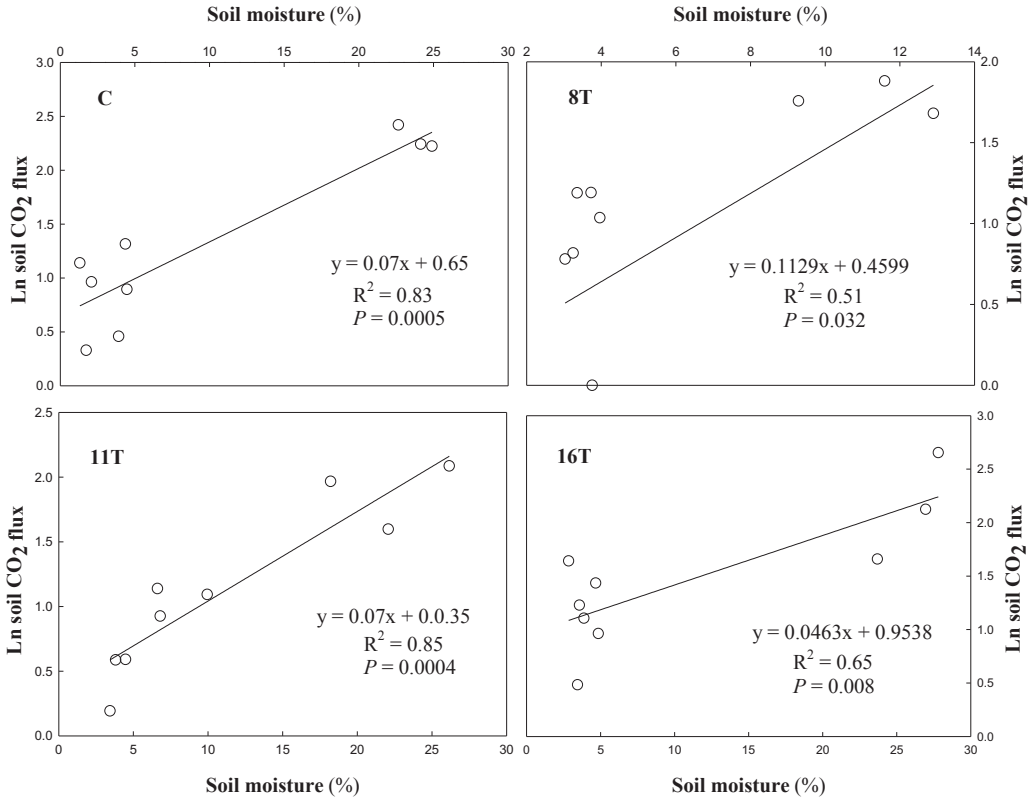

Fig. 7. Relationship of soil CO_2 flux (μ mole CO_2 m^{-2} s) with soil moisture in different precipitation regime (Treatments i.e. IG, NIG and B were pooled across two yearly seasonal data sets of 2015–16).

Table 3
Pearson correlation coefficients between soil CO_2 flux, soil moisture and soil temperature in different precipitation regime under ambient (open C) and rainout sheltered plots (8T, 11T, 16T).

	Open C			800 (8T)			1100 (11T)			1600 (16T)		
	IG	NIG	B	IG	NIG	B	IG	NIG	B	IG	NIG	B
Soil Moisture	**0.71****	**0.76****	**0.53****	**0.36***	**0.35***	**0.63****	**0.74****	**0.77***	**0.61****	**0.73****	**0.78****	**0.73****
Soil Temperature	**0.35***	0.14 ^{NS}	**0.60****	**0.34***	**0.41***	**0.08****	0.14 ^{NS}	0.24 ^{NS}	−0.14 ^{NS}	0.24 ^{NS}	0.24 ^{NS}	0.24 ^{NS}

Data of entire thirteen months were taken (Dec-2015 to Dec-2016).
NS = Not significant.
* Significance at P < 0.05.
** Significance at P < 0.01.

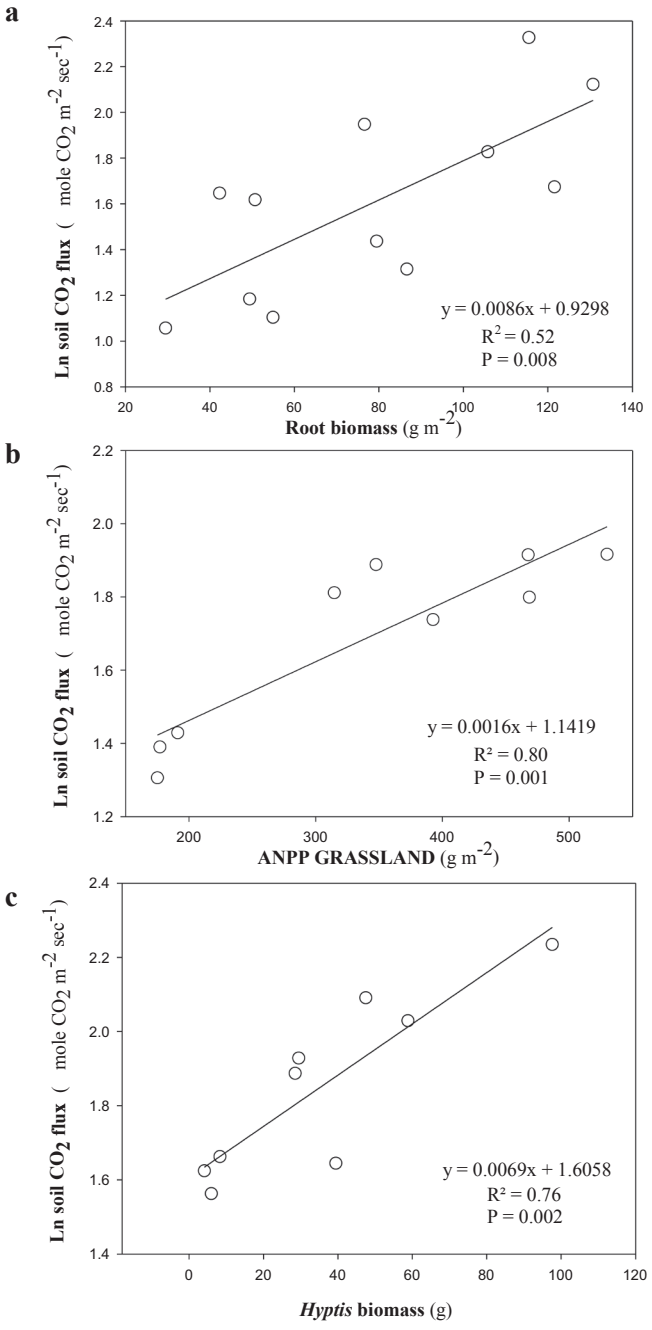
a
Ln soil CO2 flux (mole CO2 m-2 sec-1)
y = 0.0086x + 0.9298
R2 = 0.52
P = 0.008
Root biomass (g m-2)
b
Ln soil CO2 flux (mole CO2 m-2 sec-1)
y = 0.0016x + 1.1419
R² = 0.80
P = 0.001
ANPP GRASSLAND (g m-2)
c
Ln soil CO2 flux (mole CO2 m-2 sec-1)
y = 0.0069x + 1.6058
R² = 0.76
P = 0.002
Hyptis biomass (g)

Fig. 8. Relationship of soil CO_2 flux with (a) root biomass (average data of root biomass in different precipitation were used) (b) ANPP of grassland (replicate data of September month in NIG plots were used) (c) *Hyptis* biomass (replicate data of September month in IG plots were used).

Table 4
Regression equation based on soil moisture (SM), root biomass (RB) and soil temperature (ST) in sheltered plots for the prediction of soil CO_2 flux.

Soil CO_2 flux = 1.19 (± 0.13) + 0.04 (± 0.1) (SM)	[R^2 = 0.61]	[P = 0.003]
Soil CO_2 flux = 0.92 (± 0.17) + 0.03 (± 0.1) (SM) + 0.01 (± 0.002) (RB)	[R^2 = 0.74]	[P = 0.002]
Soil CO_2 flux = 0.44 (± 0.39) + 0.03 (± 0.01) (SM) + 0.07 (± 0.003) (RB) + 0.01 (± 0.01) (ST)	[R^2 = 0.79]	[P = 0.004]

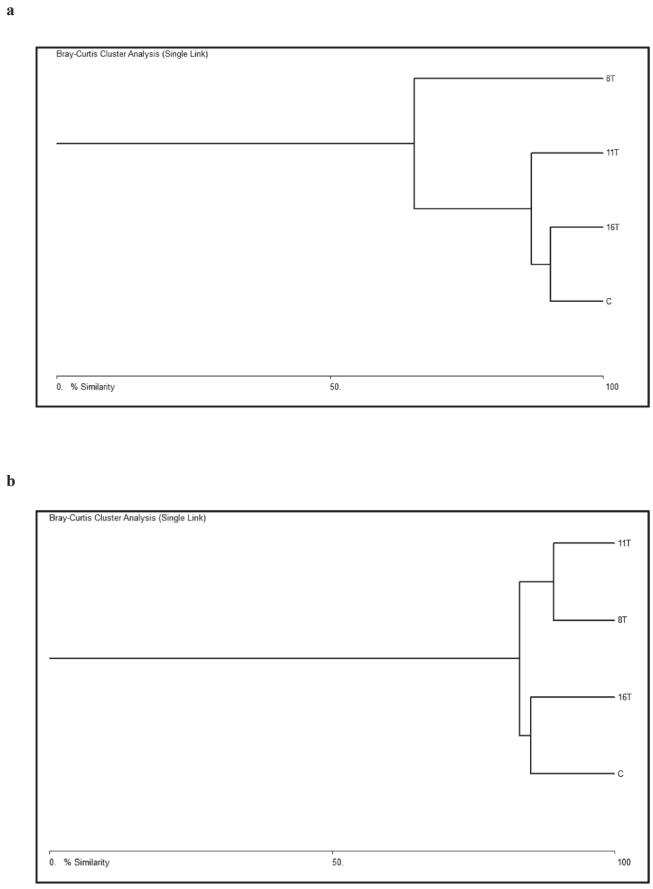

Fig. 9. Cluster analysis of the precipitation treated plots (8T, 11T, 16T) along with the ambient plots (open C). On the basis of (a) soil moisture and (b) soil CO_2 emission from the respective plots. (Treatments i.e. IG, NIG and B were pooled across two yearly seasonal data sets of 2015–16).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.02.022.

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